UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/702,507	11/07/2003	Jacques Degelaen	Neogen 4.1-48	8535

21036 7590 12/17/2007

MCLEOD & MOYNE, P.C.
2190 COMMONS PARKWAY
OKEMOS, MI 48864

EXAMINER	
NGUYEN, BAO THUY L	
ART UNIT	**PAPER NUMBER**
1641	
MAIL DATE	**DELIVERY MODE**
12/17/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/702,507
Filing Date: November 07, 2003
Appellant(s): DEGELAEN ET AL.

Ian C. McLeod
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 19 September 2007 appealing from the Office action mailed 06 March 2007.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

6,319,466	Markovsky et al	11-2001
6,074,869	Pall et al	6-2000

Joris et al., FEMS Microbiology Letters. Vol. 70. No. 1. 15 June 1990. Pages 107-113.

EP 0 093 613 Litman et al. (09 November 1983).

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 24, 26-32 and 36-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Markovsky et al (US 6,319,466) in view of Joris (*FEMS Microbiology Letters*. Vol. 70. No. 1. 15 June 1990. Pages 107-113) and Litman et al (EP 0 093 613).

Markovsky discloses a device comprising a labeled receptor positioned within or proximate to a membrane. The membrane comprises a test zone having an analyte

conjugate immobilized thereto to bind unbound receptor to form a first analyte conjugate receptor complex. The membrane further comprising a control zone including a binder immobilized thereto. See column 1, lines 52-67. Markovsky teaches that the receptor may bind a family of analytes which have similar structural binding sites. Markovsky also discloses a sample absorbing and a mobile-phase support zone acting as a filter for somatic cells. See column 9, lines 7-14. The mobile-phase support zone is preferably Porex® pad or Porex® Lateral Flow Media (a rigid pore structure made from high density polyethylene). See column 10, lines 25-29. The device is configured to detect analytes such as beta lactams antibiotics in milk samples. See column 5, lines 11-20. The entire device is provided in a blister package including a removable seal strip at one end for application of the sample. See column 4, lines 8-20. Markovsky teaches that competitive assays for beta-lactams in milk sample can be done in 2 to 15 minutes. See column 3, lines 26-32. Markovsky teaches that test kits for detecting beta-lactams in biological fluids are well known in the art. See column 1.

Markovsky differs from the invention in failing to teach receptors obtained from *Bacillus lichenformis* as the labeled reagent. Markovsky also fails to teach a reference that is independent of the analyte.

Joris, however, discloses BLAR and BLAR-CTD involved in β-lactamase inducibility in *Bacillus lichenformis.*

And, Litman discloses a method and device for detecting an analyte comprising a measurement surface and a calibration surface binding to a reagent independent from the analyte. See page 4, lines 24-37.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use the receptors taught by Joris in the device of Markovsky because Markovsky teaches that their device can be modified for the detection of a variety of analytes, including those disclosed in claim 32, using appropriate reagents. See column 13, lines 23-39. Markovsky teaches that most of the elements for each test are the same except the chemistries of the mobile phase, test zone and control zone, which are tailored to the specific analyte detection. Since Joris discloses that receptors of as BLAR and BLAR-CTD are readily available ~~are~~ and well known in the art as having β-lactamase activity, a skilled artisan would have had a reasonable expectation of success in using receptors BlaR or BlaR-CTD to detect beta-lactams antibiotics such as penicillin as taught by Markovsky.

Even though Markovsky does not specifically teaches that the mobile-phase support zone (i.e. purification membrane) retains leukocytes, Markovsky teaches that this zone is capable of filtering somatic cells, therefore, a skilled artisan would have had a reasonable expectation of success that such a membrane is capable of retaining leukocytes.

The use of an independent reference or calibration reagent is well known in the art and a skill artisan would have been motivated to use the calibration method and reagents taught by Litman in the device of Markovsky because Markovsky teaches a control comprising a broad spectrum antibody that is captured at the reference zone regardless of the presence or absence of an analyte in a sample and Litman teaches that it is advantageous to use a reagent that is independent from the analyte to provide a calibration or reference signal such that a standard for evaluation of the analyte at the detection zone can be obtained.

Claims 34 and 35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Markovsky in view of Joris and Litman as applied to claim 24 above, and further in view of Pall et al (US 6,074,869).

See the discussion of Markovsky, Joris and Litman above. These references differ from the instant invention in failing to specifically disclose the pore size of the purification membrane.

Pall, however, teaches membranes for filtering biological samples, including leukocytes and milk sample. See column 6, lines 32-62. Pall teaches that their membrane is a non-woven web (i.e. polyethylene) having an average pore size of 3 to 8μm. See column 8, lines 54-60.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the purification membrane taught by Pall in the device of Markovsky as modified by Joris because such a membrane is well known in the art and provides the advantage of a substantially uniform porous medium that can separate large somatic cells from a biological sample.

Response to Arguments

Appellant's arguments have been fully considered but they are not persuasive. Appellant argues that the prior art rejection is based upon the premise that it would be "obvious to try" to adapt the BlaR or BlaR-CTD proteins of Joris to the lateral flow assay of Markovsky even though Joris does not suggest any assays at all.

This argument is not persuasive. Markovsky teaches a device specifically for detecting beta lactam antibiotics in milk samples such as claimed. Markovsky teaches the detection of a variety of analytes including penicillin, ampicillin, ceftiofur, etc (column 13, lines 25-40) using reagents tailored specifically to the analyte. As such, it is entirely within the skills of the ordinary artisan to choose a receptor that is specific for the analyte, and since Joris teaches that receptors to penicillin, BlaR and BlaR-CTD, are readily available and are well known in the art as having β-lactamase activity, it would have been obvious for one of ordinary skill in the art to use these receptors in the device of Markovsky to detect penicillin in milk samples.

Appellant argues that in a complex lateral flow assay as claimed, there would be no assurance that the BlaR or Blar-CTD receptors would function since the complex with the antibiotic is subjected to lateral flow and different proteins have different flow characteristics, therefore, one skilled in the art could not predict that the BlaR or BlaR-CTD receptors could function in this manner.

This argument is not persuasive. Lateral flow assays are well known in the art and Markovsky recognizes the need to test the mobility of sample such as milk to optimize reaction times and uniformity. Markovsky discloses that high pore size membranes (15 to 140 μm) are used to allow flow of viscous samples like milk or serum (column 11, lines 64-67). Therefore, there is clear guidance for an ordinary artisan to test the mobility of the reagents in an assay to optimize reaction times and uniformity as taught by Markovsky. Furthermore, Markovsky teaches the detection of beta-lactams antibiotic in milk samples just as those in the instant claims. Markovsky teaches the use of beta lactam receptors purified from bacteria, and since BlaR and BlaR-CTD are also beta-lactam receptors purified from bacteria, these receptors would be expected to be similar to those disclosed by Markovsky and would also be expected to have similar characteristics, therefore, the simple substitution of the beta-lactam receptors taught by Markovsky with the beta-lactam receptors of Joris would be expected to yield predictable results. There is every expectation that the beta-lactam receptors taught by Joris would be able to flow through the device of Markovsky since these receptors are similar.

Appellant argues that there is no suggestion that BlaR or BlaR-CTD bound with the antibiotics would pass through the web described by Pall. One skilled in the art would have no basis for suggesting that the Pall fibrous web could be used in the claimed lateral flow assay kit for dairy products.

This argument is not persuasive. Pall teaches membranes for filtering biological samples including leukocytes. See column 6, lines 32-62. Pall specifically teaches polyethylene membrane having pore size of 3 to 8 μm. See column 8, lines 54-60 and column 18, lines 17-27. Since this membrane is similar to that which is taught by Markovsky (i.e. polyethylene membrane), and since the membrane of Markovsky has been shown to be capable of supporting the flow of BlaR or BlaR-CTD bound to antibiotic as argued above, one of ordinary skill in the art would have had a reasonable expectation of success in using the membrane taught by Pall in the device of Markovsky for the advantage of a substantially uniform porous medium that can separate large somatic cells from a biological sample. Furthermore, the membrane taught by Pall has the same pore size as that of the instant claims; therefore, this membrane would inherently possess the same characteristics as the claimed membrane. If the claimed membrane with its 8 μm pore size can support the flow of BlaR or BlaR-CTD bound to antibiotic, than the membrane of Pall with its 8μm pore size can also support the same.

For the above reasons, it is believed that the rejections should be sustained.

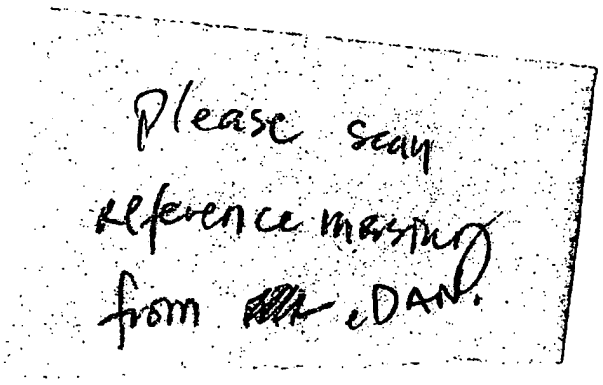
Respectfully submitted,

BAO-THUY L. NGUYEN
PRIMARY EXAMINER
11/06/07

Conferees:

LONG V. LE
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

ROBERT A. WAX
PRIMARY EXAMINER
TQAs Appeals Specialist


Please scan
reference mapping
from [redacted] eDAN.

FEMS Microbiology Letters 70 (1990) 107–114
Published by Elsevier

FEMSLE 04051

Expression in *Escherichia coli* of the carboxy terminal domain of the BLAR sensory-transducer protein of *Bacillus licheniformis* as a water-soluble M_r 26000 penicillin-binding protein

B. Joris [1], P. Ledent [1], T. Kobayashi [2], J.O. Lampen [2] and J.M. Ghuysen [1]

[1] *Service de Microbiologie, Université de Liège, Institut de Chimie, B6, Liège, Belgium and* [2] *Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, NJ, U.S.A.*

Received 24 January 1990
Revision received 9 March 1990
Accepted 14 March 1990

Key words: β-Lactamase induction; *Escherichia coli*; *Bacillus licheniformis*; BLAR-CTD

1. SUMMARY

A cloning vector has been constructed which allows production and export by *Escherichia coli* of the Met346-Arg601 carboxy terminal domain of the 601 amino acid BLAR sensory-transducer involved in β-lactamase inducibility in *Bacillus licheniformis*. The polypeptide, referred to as BLAR-CTD, accumulates in the periplasm of *E. coli* in the form of a water-soluble, M_r 26000 penicillin-binding protein. These data and homology searches suggest that BLAR has a membrane topology similar to that of other sensory-transducers involved in chemotaxis.

2. INTRODUCTION

As previously reported [1,2], BLAR appears to be a two-domain protein with an amino terminal domain that consists of several transmembrane segments and a carboxy terminal domain that protrudes on the outer face of the membrane and serves as penicillin receptor. On the basis of this presumed membrane topology and given that the penicillin receptor per se, i.e. BLAR-CTD, has high similarity, in the primary structure, with the class D Oxa-2 β-lactamase of *Salmonella typhimurium* [2], a cloning vector was constructed in which that portion of the *bla*R gene encoding BLAR-CTD was placed under the control of the promoter, ribosome-binding site and signal peptide-encoding sequence of the Oxa-2 β-lactamase. The expectation was that *Escherichia coli* harbouring the resulting combinant plasmid pDML307 would export BLAR-CTD in the form of a novel, water-soluble penicillin-binding protein.

3. MATERIALS AND METHODS

Plasmid pRTW8 [1] was the source of the *bla*R gene, plasmid R46 [3] was the source of the gene encoding the Oxa-2 β-lactamase and plasmid

Correspondence to: J.M. Ghuysen, Service de Microbiologie, Université de Liège, Institut de Chimie, B6, B-4000 Sart Tilman (Liège 1), Belgium.

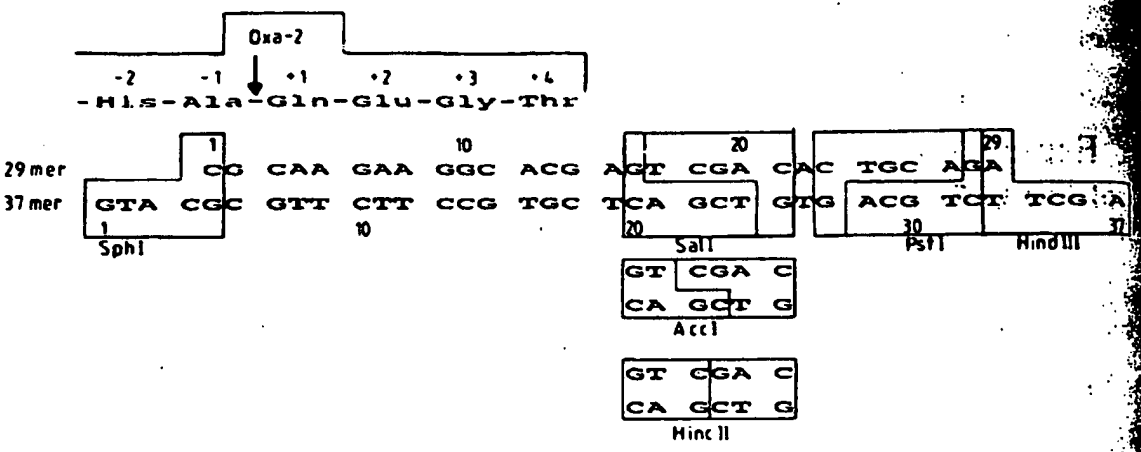


Fig. 1. Nucleotide sequence of the polylinker. The nucleotides 1 to 14 of the 29-mer were designed to reconstitute the −1 to +6 amino acid sequence of the Oxa-2 β-lactamase. The arrow indicates the site of cleavage of the β-lactamase precursor by the leader peptidase. The 29-mer–37-mer hybrid provides cohesive ends specific of *Sph*I and *Hin*dIII.

pBGS18$^+$ [4] was used as vector. pBGS18$^+$ contains the multiple cloning region of M13mp18 inserted in the 5′ end of the *lacZ′* gene and has the *Km*R gene conferring resistance to kanamycin. *E. coli* JM105 (*lac*$^-$, *proAB*, *thi*, *rpsL*, *endA*, *sbc*B15, *hspR4* [F′, *pro*$^+$, B$^+$, *lac*I^{q}Z M15, *tra*D]) [5] was used as host. Standard DNA recombinant [6] and nucleotide sequencing [7] techniques were used. The polylinker shown in Fig. 1 was synthesized with a DNA synthesizer Biosearch Cyclone (New Brunswick Scientific Co., San Raphael, CA, USA). Bacterial growth was carried out at 37°C in liquid YT medium [8] and on 2 × YT agar plates. Selection of kanamycin- and ampicillin-resistant strains was made on agar plates containing 25 μg antibiotic ml^{-1}. Detection of *lacZ′* α-complementation was made on agar plates containing per ml 100 μg 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and 40 μg isopropyl-β-D-thiogalactopyranoside.

Cell fractionation was carried as described in [9]; β-galactosidase and β-lactamase activities were assayed on *o*-nitrophenyl-β-galactopyranoside [10] and nitrocefin [11], respectively; DD-carboxypeptidase (reaction catalysed: Ac_2-L-Lys-D-Ala-D-Ala + H_2O → Ac_2-L-Lys-D-Ala + D-Ala) and DD-transpeptidase (Ac_2-L-Lys-D-Ala-D-Ala + [^{14}C]D-Ala → Ac_2-L-Lys-D-Ala-[^{14}C]D-Ala + D-Ala) activities were measured and penicillin binding (using [^{35}S]benzylpenicillin) was carried as described in [12,13].

pDML307 (see INTRODUCTION) was constructed as indicated in Fig. 2 Subcloning of the Oxa-2 β-lactamase was made by inserting the 2.5 kb *Pst*I 50860-*Bam*HI 1160 DNA fragment of R46, in the *Bam*HI-*Pst*I site of the multiple-cloning region of pBGS18$^+$ (arrow). The ligation mixture was used to transform *E. coli* JM105. Ampicillin-resistant and kanamycin-resistant transformants were selected. The isolated and purified recombinant plasmid pDML303 contained: (i) from *Bam*HI 170 to *Sph*I 1670 (with an additional internal *Sph*I site 1030) and downstream of *Ava*I 1500, the promotor, the ribosome-binding site and the sequence coding for the signal peptide of the Oxa-2 β-lactamase; and (ii) from *Sph*I 1670 to *Hin*dIII 2670 the rest of the Oxa-2 β-lactamase gene. The next step was therefore to replace this latter piece of DNA by the *bla*R-CTD gene which, in pRTW8, extends from *Bal*I 4360 to *Hin*dIII 5590. This was achieved as follows. (i) The *Sph*I 1030 - *Hin*dIII 2670 DNA fragment was excised from pDML303 and replaced by the *Sph*I*Hin*dIII polylinker of Fig. 1, yielding plasmid pDML304. Note that the nucleotides 1 to 14 of the 29-mer strand of the polylinker were designed to recon-

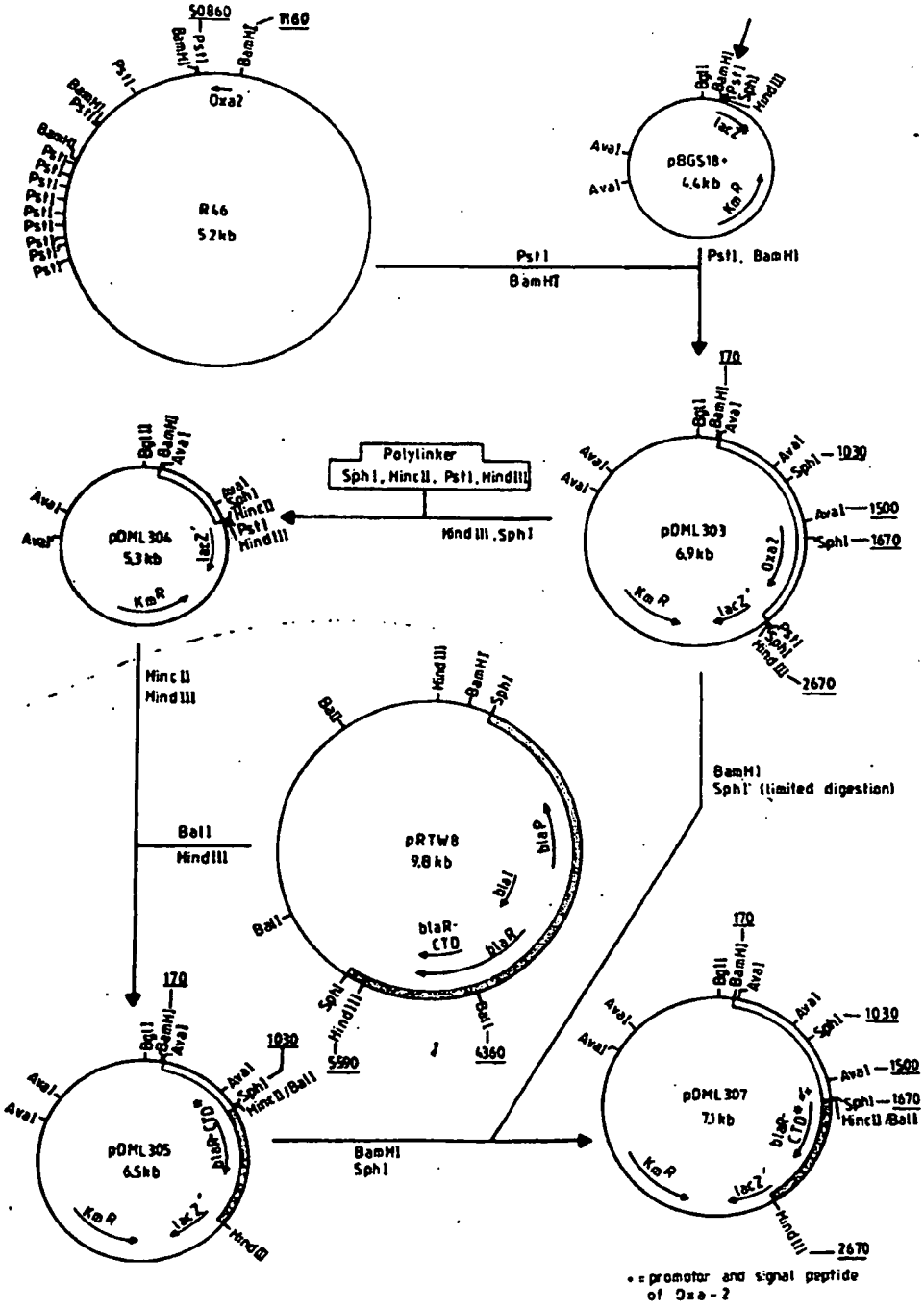


Fig. 2. Construction of plasmid pDML307.

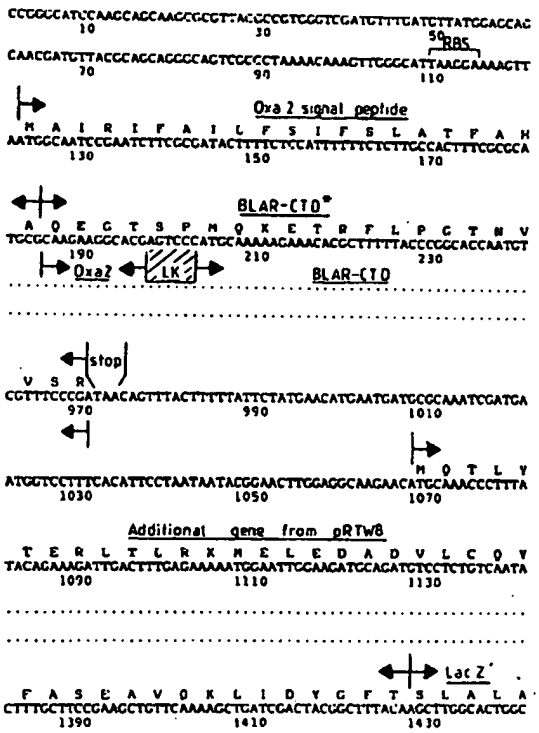


Fig. 3. Organization of the *bla*R-CTD* gene in pDML307. The promoter, the ribosome-binding site (RBS) and the 75 base-pair sequence coding for the signal peptide and the tetrapeptide QEGT of the amino terminal region of the Oxa-2 β-lactamase were linked to the *bla*R-CTD gene via a 6-base-pair sequence (originating from the linker and coding for the dipeptide SerPro). The *bla*R-CTD gene was followed by another *B. licheniformis* gene (from pRTW8) itself in frame with the *lacZ'* gene.

stitute the −1 to +4 sequence of the Oxa-2 β-lactamase, i.e. the sequence AQEGT containing the AQ site of cleavage by the leader peptidase. (ii) The polylinker in pDML304 was digested with *Hin*cII and *Hin*dIII thus providing the sites for the insertion of the *Bal*I 4360–*Hin*dIII 5590 DNA fragment of pRTW8 that contained the *bla*R-CTD gene. The resulting plasmid was called pDML305. iii) The *Bam*HI 170–*Sph*I 1030 DNA fragment of pDML305 was excised and replaced by the *Bam*HI 170–*Sph*I 1670 DNA fragment of pDML303. The recombinant plasmid was called pDML307.

4. RESULTS

The organization of the insert in pDML307 is shown in Fig. 3. pDML307 was used to transform *E. coli* JM105 and the resulting strain was called *E. coli* JM105/pDML307. Given the site of cleavage by the leader peptidase (vertical arrow in Fig. 3), *E. coli* JM105/pDML307 was expected to produce and export into the periplasm, a 262 amino acid polypeptide referred to as BLAR-CTD*—i.e. BLAR-CTD with a QEGTSP hexapeptide amino terminal extension. Since this small extension should not interfere with the folding of the polypeptide, the exported BLAR-CTD* was

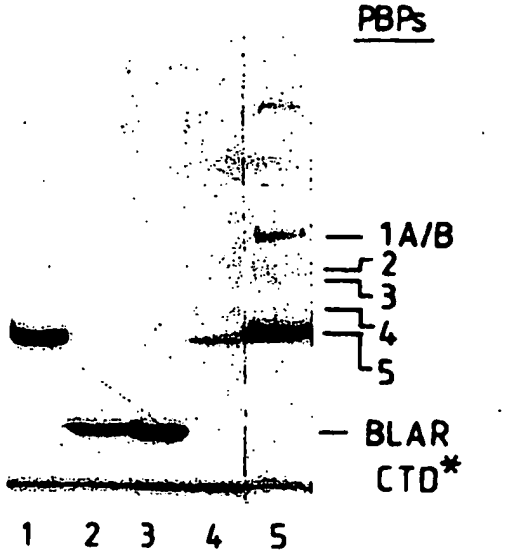


Fig. 4. Periplasmic location of BLAR-CTD* expressed by *E. coli* JM105/pDML307. The periplasmic fraction (8 and 60 μg protein; lanes 2 and 3, respectively), the cytoplasmic fraction (175 μg protein; lane 4) and the plasma membrane fraction (20 μg protein; lane 5) were labelled with [^{35}S]benzylpenicillin, submitted to SDS–polyacrylamide gel electrophoresis and the radioactively labelled penicillin-binding proteins were visualized by fluorography. The 38000 M_r DD-peptidase/penicillin binding proteins of *Streptomyces* R61 (5 pmol; lane 1) used as standard was similarly treated. The cytoplasmic fraction (lane 4; 175 μg protein) is slightly contaminated by membrane components. Lane 5 shows the membrane-bound penicillin-binding proteins (PBPs), from PBPs 1A/1B (molecular weight: 93636 and 94266, respectively) to PBP5 (molecular weight: 44444), of the host *E. coli* JM105.

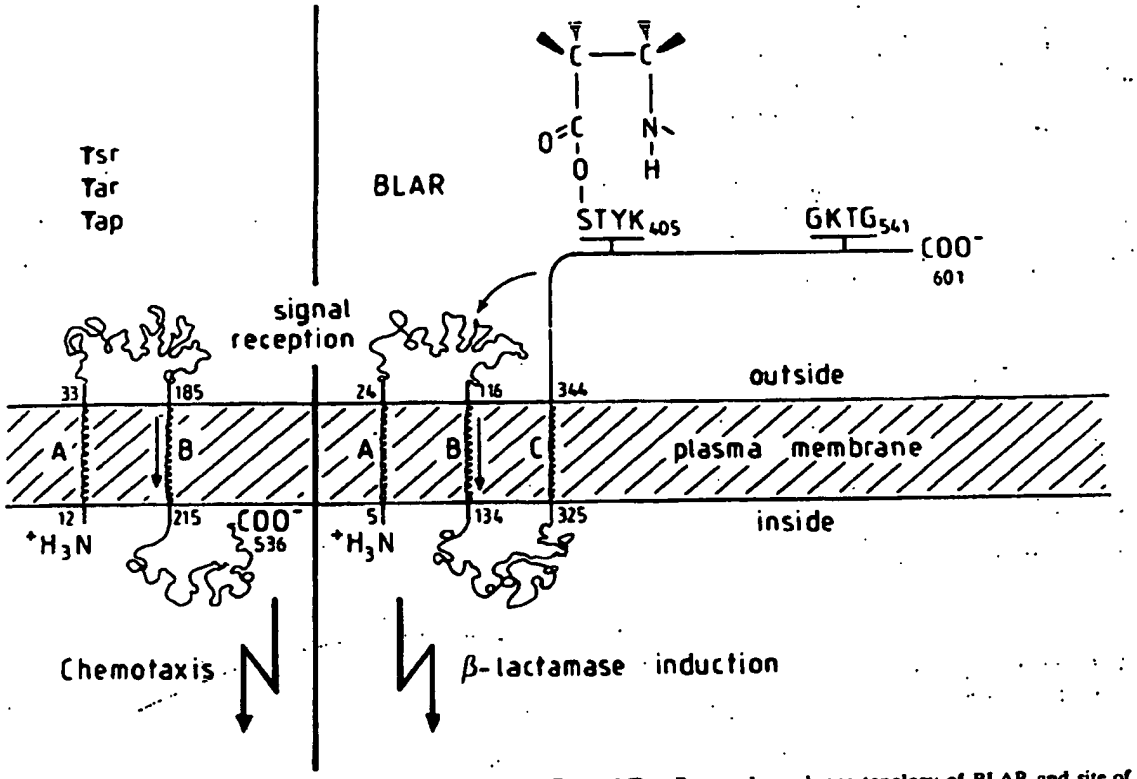


Fig. 5. Membrane topology of the chemotactic transducers Tsr, Tar and Tap. Proposed membrane topology of BLAR and site of penicilloylation.

also expected to behave as a 26 000-M_r penicillin-binding protein. Finally, *E. coli* JM105/pDML307 was expected to be a producer of intracellular β-galactosidase (as a result of an in-frame fusion between an additional gene from pRTW8 and *lacZ'*) and of extracellular β-lactamase (given that the host *E. coli* JM105 had the chromosome-encoded *amp*C gene).

Analysis of the penicillin-binding proteins in isolated cell fractions revealed that *E. coli* JM105/pDML307 produced during growth, a water-soluble, 26 000-M_r penicillin-binding protein that accumulated exclusively in the periplasm (Fig. 4). Using the *Streptomyces* R61 DD-peptidase/penicillin-binding protein as reference [14], one overnight culture of *E. coli* JM105/pDML307 contained about 1 mg BLAR-CTD* per litre of culture. The periplasmic fraction lacked detectable DD-carboxypeptidase of DD-transpeptidase activity, suggesting that the only property of BLAR-CTD* was to bind penicillin. Analysis of the *E. coli* JM105/pDML307 cell fractions also showed that 80% of the expressed β-galactosidase was cytoplasmic and 80% of the expressed β-lactamase was periplasmic.

Note that the *E. coli* JM105 strains harbouring pBGS18⁺, pDML303 or pDML304 did not produce any periplasmic penicillin-binding protein and that *E. coli* JM105/pDML305 produced trace amounts of BLAR-CTD* (presumably intracellularly). Given that immediately downstream of the *Bal*I site, there occurs and initiation codon ATG,

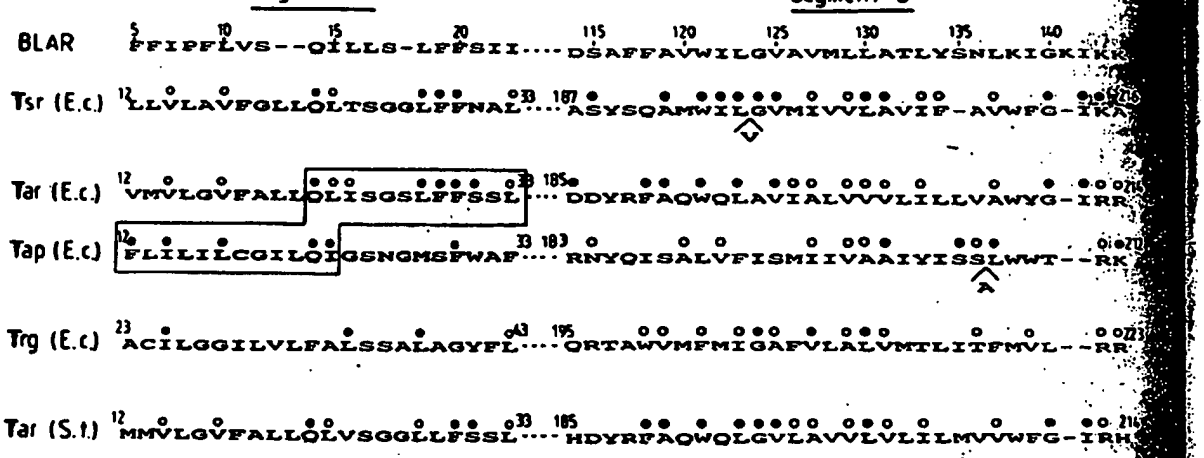


Fig. 6. Alignment of the 5-23 amino acid segment and the 114-144 amino acid segment of BLAR with the transmembrane segments and neighbouring amino acids, of the Tsr, Tar, Tap and Trg sensory transducer proteins of Gram-negative bacteria. E.c = *E. coli*; S.t = *Salmonella typhimurium*; ● = identities; ○ = conservative replacements; ∧ = insertions. The transducers are involved in chemotactic responses to serine (Tsr), aspartate and maltose (Tar), galactose and ribose (Trg) and dipeptides (Tap). A and B are transmembrane segments (Fig. 5).

this low expression indicated the presence of a promoter in the *Bam*HI 170-*Sph*I 1030 DNA segment.

5. DISCUSSION

Transducers are transmembrane proteins specialized in the transmission of a chemical signal from the environment to the interior of the bacterial cell. They contain an extracellular domain which is located on the outer face of the membrane and is responsible for signal reception, and a cytosolic domain which is responsible for the generation of an intracellular signal (Fig. 5). In chemotaxis, the Tsr, Tar, Trg and Tap transducers are sensitive to amino acids (serine, aspartate), carbohydrates (maltose, galactose, ribose) and dipeptides [15]. Attractants either bind directly to the transducer or, alternatively, they bind first to soluble binding proteins (located in the periplasm of Gram-negative bacteria) and the attractant: attractant-binding protein then binds to the transducer.

Amino acid alignments (Fig. 6) revealed that the polypeptide segment D115-K143 of BLAR is very similar, in its primary structure, to the transmembrane segment B which links the receptor domain to the cytoplasmic domain of Tsr in *E. coli* (12 strict identities out of 20 amino acids) or Tar in *Salmonella typhimurium* (9 strict identities). Also, the polypeptide segment F5-S21 of BLAR exhibits similarity with the transmembrane segment A of Tar and Tap of *E. coli*. On the basis of these homologies and the fact that, as shown above, BLAR-CTD is the penicillin-binding domain of BLAR, a likely molecular organization of BLAR is that shown in Fig. 5. The 325 amino acid amino terminal region, i.e. the transducer per se, would have the same general membrane topology as that found in the chemotactic transducers Tsr, Tar and Tap. As observed with some transducers, the attractant of BLAR, i.e. penicillin, does not bind directly to the transducer but binds to the 257 amino acid penicillin-binding BLAR-CTD. The peculiarity of BLAR is that the BLAR-CTD is fused to the carboxy terminus of the transducer by means of an additional transmembrane segment C, which fusion is a direct consequence of the absence of periplasm in *B. licheniformis*.

One may hypothesize that penicilloylation of BLAR-CTD induces conformational changes and that this signal is transmitted to the extracellular domain of the transducer, and from this, to the intracellular domain via the transmembrane segment B. In agreement with this view, the Gly124 → Asp and Gly538 → Asp BLAR mutants are desensitized to penicillin and fail to induce β-lactamase synthesis in *B. licheniformis* (in preparation). The mutation Gly124 → Asp affects the transmembrane segment B and the mutation Gly538 → Asp occurs upstream of the KTG box which, in the active-site serine, penicillin-interactive proteins of known three-dimensional structure, occurs on the innermost strand of the five-stranded β-sheet and form part of the active-site [19–22]. The intracellular domain of BLAR has no multiple sites of methylation/demethylation as found in the chemotactic transducers [16] (though a dyad Glu-Glu occurs at positions 163 and 164); it lacks sequences that would suggest the occurrence of ATP binding sites [23]; it does not contain the sequence consensus characteristic of transmitters [24], but it contains five cysteine residues at positions 161, 208, 217, 229 and 256.

ACKNOWLEDGEMENTS

The work in Liège was supported in part by the Fonds de la Recherche Scientifique Médicale (contract no. 3.4507.83), an Action concertée with the Belgian Government (convention 86/91-90), the Fonds de Recherche de la Faculté de Médecine ULg and a contract with the EEC (BAP-0197-B). The work at Rutgers was supported in part by Public Health Service Grant AI-23096 from the National Institute for Allergy and Infectious Diseases and by the Charles and Johanna Busch Fund.

REFERENCES

[1] Kobayashi, T., Zhu, Y.F., Nicholls, N.J., and Lampen, J.O. (1987) J. Bacteriol. 169, 3873–3878.
[2] Zhu, Y.F., Curran, I.H.A., Joris, B., Ghuysen, J.M., and Lampen, J.O. (1990) J. Bacteriol. 72, 1137–1141.
[3] Brown, A.M.C., Coupland, G.M., and Willets, N.S. (1984) J. Bacteriol. 159, 472–481.
[4] Spratt, B.G. Hedge, P.J., te Heesen, S., Edelman, A., and Broome-Smith, J.K. (1986) Gene 41, 337–342.
[5] Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene 33, 103–109.
[6] Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.
[7] Sanger, F., Nicklen, S., and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5463–5467.
[8] Miller, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
[9] Linquist, S., Galleni, M., Lindberg, F. and Normark, S. (1989) Mol. Microbiol. 3, 1091–1102.
[10] Craven, G.R., Steers, E., and Anfinsen, C.B. (1965) J. Biol. Chem. 240, 2468–2478.
[11] O'Callaghan, C.H., Morris, A., Kerby, S.M., and Shingler, A.H. (1972) Antimicrob. Agents Chemother. 1, 283–288.
[12] Nguyen-Distèche, M., Leyh-Bouille, M., Pirlot, S., Frère, J.M. and Ghuysen, J.M. (1986) Biochem. J. 235, 167–176.
[13] Leyh-Bouille, M., Nguyen-Distèche, M., Pirlot, S., Veithen, A., Bourguignon, C. and Ghuysen, J.M. (1986) Biochem. J. 235, 177–182.
[14] Duez, C., Piron-Fraipont, C., Joris, B., Dusart, J., Urdea, M.S., Martial, J.A., Frère, J.M., and Ghuysen, J.M. (1987) Eur. J. Biochem. 162, 509–518.
[15] MacNab, R.M. (1987) in *Escherichia coli* and *Salmonella typhimurium* cellular and molecular biology, Vol. 1 (F.C. Neidhardt, J.L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaechter and H.E. Umbarger, eds.). pp. 732–759, American Society for Microbiology, Washington, D.C.
[16] Bollinger, J., Park, C., Harayama, S., and Hazelbauer, G.L. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 3287–3291.
[17] Krikos, A., Mutoh, N., Boyd, A., and Simon, M.I. (1983) Cell 33, 615–622.
[18] Russo, A.F., and Koshland Jr, D.E. (1983) Science 220, 1016–1020.
[19] Herzberg, O., and Moult, J. (1987) Science 236, 694–701.
[20] Dideberg, O., Charlier, P., Wéry, J.P., Dehottay, P., Dusart, J., Erpicum, T., Frère, J.M. and Ghuysen, J.M. (1987) Biochem. J. 245, 911–913.
[21] Joris, B., Ghuysen, J.M., Dive, G., Renard, A., Dideberg, O., Charlier, P., Frère, J.M., Kelly, J.A., Boyington, J.C., Moews, P.C., and Knox, J.R. (1988) Biochem. J. 88, 313–324.
[22] Kelly, J.A., Knox, J.R., Zhao, H., Frère, J.M., and Ghuysen, J.M. (1989) J. Mol. Biol., 209, 281–295.
[23] Walker, J.E., Saraste, M., Runswick, M.J., and Gay, N.J. (1982) EMBO J. 1, 945–951.
[24] Kofoid, E.C., and Parkinson, J.S. (1988) Proc. Natl. Acad. Sci. USA 85, 4981–4985.